

Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content

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Abstract Phytate (inositol-hexa-phosphate) has an important role in plants but it also may have anti-nutritional properties in animals and humans. While there is debate within the plant breeding and nutrition communities regarding an optimum level in grain, there appears to be little information at the molecular level for the genetics of this trait, and its association with important trace elements, in particular, Fe and Zn. In this preliminary study, quantitative trait loci (QTL) for grain phytates, Zn and Fe in glasshouse-grown rice lines from an IR64 × Azucena doubled haploid population were identified. Correlations between phytate and essential nutrients were also studied. Transgressive segregation was found for most traits. Phytate and total P concentrations had one QTL in common located on chromosome

five with the (high concentration) allele contributed from Azucena. There were significant positive correlations between phytate and inorganic phosphorus (P), total P, Fe, Zn, Cu and Mn concentrations for both grain concentration and content. However, the QTLs of phytate were not located on the same chromosomal regions as those found for Fe, Zn and Mn, suggesting that they were genetically different and thus using molecular markers in breeding and selection would modify the phytate level without affecting grain micronutrient density.

Keywords Iron · Micronutrients · Nutrition · Phytate · Quantitative trait loci · Rice

Introduction

Human nutritionists regard phytate (phytic acid or phytin) as a major anti-nutrient present in legume seeds and cereal grains, reducing the bio-availability of dietary Zn and non-heme Fe. However, reducing the level of phytate in plant foods is still under debate. While breeding for low phytate genotypes has become a nutritional objective (Raboy 2001, 2002), phytate possesses a number of valuable properties essential for both plant and human health. For example, seed phytate is a major source of P that supports seedling growth on P-deficient soil (Marshner 1995).

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Phytic acid may play important roles in lowering chronic disease rates in humans, including certain types of cancers and heart diseases (Graham et al. 2001). Welch (2002) suggests that breeding for low phytate in food crops is not without risk and should be done with caution.

Understanding the genetics of phytate accumulation in grains is necessary for a breeding program. Recent research has identified QTL affecting the quantity of phytate in seeds and leaves of *Arabidopsis thaliana* (Bentsink et al. 2003). No such result has been published for rice, which is one of the most important staple cereal crops in Asia and worldwide. Our objective is to map this trait using the IR64 × Azucena doubled haploid population, to study correlations between essential nutrients, and to identify chromosomal regions where phytate may co-locate with essential nutrients that may impact in breeding for increased nutrient content in rice.

Materials and methods

A population of 129 doubled-haploid lines derived from a cross between the irrigated *Indica* variety IR64 and the upland *Japonica* variety Azucena (Guiderdoni et al. 1992) was used in this study. The population was grown in a controlled glasshouse environment with no nutrients limiting growth. Inositol-5-phosphate (IP5), inositol-6-phosphate (IP6, phytate), inorganic P (H_2PO_4^-) and total P content of unmilled rice was determined following extraction in 1.25% H_2SO_4 and subsequent analysis by Dionex liquid chromatography using 200 mM NaOH and deionized water as the eluents. Grain micronutrients (Fe, Zn, Cu and Mn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Zarcinas et al. 1987) using an ARL 35B ICP analyser. The nutrient concentrations are measured as g/kg (for IP5, IP6, inorganic P and total P) or mg/kg (for Fe, Zn, Cu and Mn), while nutrient contents are calculated as nanograms per seed.

A saturated linkage map for the Azecena × IR64 population has been constructed and can be accessed online (Temnykh et al. 2001). This

map consists of 437 microsatellite markers (SSRs) in addition to 145 RFLP anchor markers originally mapped on this population by Huang et al. (1994). Further details of development of SSRs and construction of the linkage map for the Azucena × IR64 population are reported in Causse et al. (1994), Panaud et al. (1996), Temnykh et al. (2000, 2001).

Histograms and normality tests (Pearson chi-square test) were used to describe the variation of the phenotypic traits while correlations were employed to examine their phenotypic association. QTLs were detected via the composite interval mapping method (CIM) performed with Windows QTL Cartographer V2.5 (Wang et al. 2005). In the CIM statistical model, up to five markers, called background markers, within 20 cM from either side of flanking markers served as covariance for estimating an interval. Such markers could affect the interval, so their inclusion in the model would give a more accurate QTL estimation compared to the simple interval mapping method (Zeng 1994). A minimum LOD (log of the odds) threshold of 3.0 was employed to indicate significance. This threshold value corresponded to the 0.05 probability level and was determined via a 1,000-time permutation test.

Results and discussion

The levels of phytate, inorganic P and micronutrients, especially Fe and Zn, in the doubled haploid population varied widely (Fig. 1) but were not normally distributed based on the normality test. Transgressive segregation was also observed which suggests multiple gene action for these traits. Phytate was significantly positively correlated with Fe, Zn, Cu and Mn, while Zn was significantly correlated with Fe and Cu (Table 1). Phytate was also highly correlated with IP5, inorganic P and especially total P ($R = 0.99$), indicating that the majority of P in grain was stored in the form of phytate.

Quantitative trait loci (QTL) for grain phytate and some micronutrients were identified (Fig. 2 and Table 2). Phytate concentration had two QTLs located on chromosomes five and 12, explaining 24% and 15% of the total phenotypic

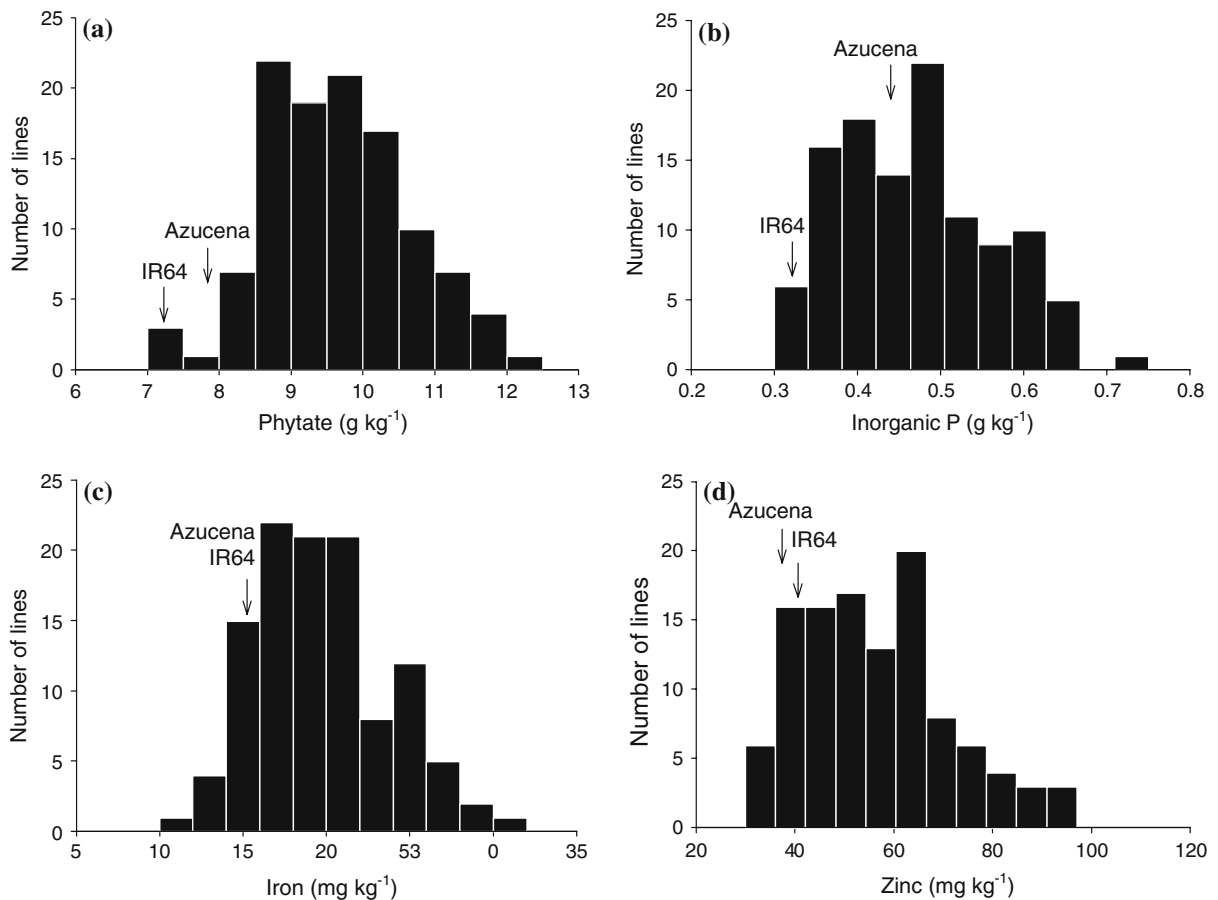


Fig. 1 Frequency distributions for grain concentration of (a) phytate (g/kg), (b) inorganic P (g/kg), (c) Fe (mg/kg) and (d) Zn (mg/kg) measured in the glasshouse grown IR64 × Azucena double haploid population

Table 1 Phenotypic correlations among grain phytates and micronutrients in the glasshouse grown IR64 × Azucena double haploid population ($n = 129$)

Trait	IP5	IP6	Inorganic P	Total P	Fe	Zn	Cu	Mn
IP5		0.341***	0.238*	0.372***	0.158	0.125	0.129	0.189*
IP6	0.279**		0.746***	0.996***	0.528***	0.402***	0.330***	0.559***
Inorganic P	0.159	0.527***		0.780***	0.431***	0.382***	0.353***	0.389***
Total P	0.327***	0.991***	0.598***		0.527***	0.405***	0.336***	0.555***
Fe	0.049	0.341***	0.189*	0.333***		0.712***	0.325***	0.346***
Zn	0.057	0.315**	0.246**	0.314**	0.691***		0.576***	0.243*
Cu	0.044	0.227*	0.233*	0.233*	0.270**	0.540***		0.227*
Mn	0.096	0.337***	0.091	0.326***	0.144	0.114	0.112	

The lower triangle indicates the nutrient concentration, and the upper triangle indicates the nutrient content

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

variation, respectively. The QTL on chromosome five was also expressed for total P concentration, explaining 20% of the phenotypic variation. Inorganic phosphate had one QTL on chromo-

some seven explaining 27% of the phenotypic variation. Iron concentration had three QTLs located on chromosomes 2, 8 and 12, explaining approximately 17%, 18% and 14% of the total

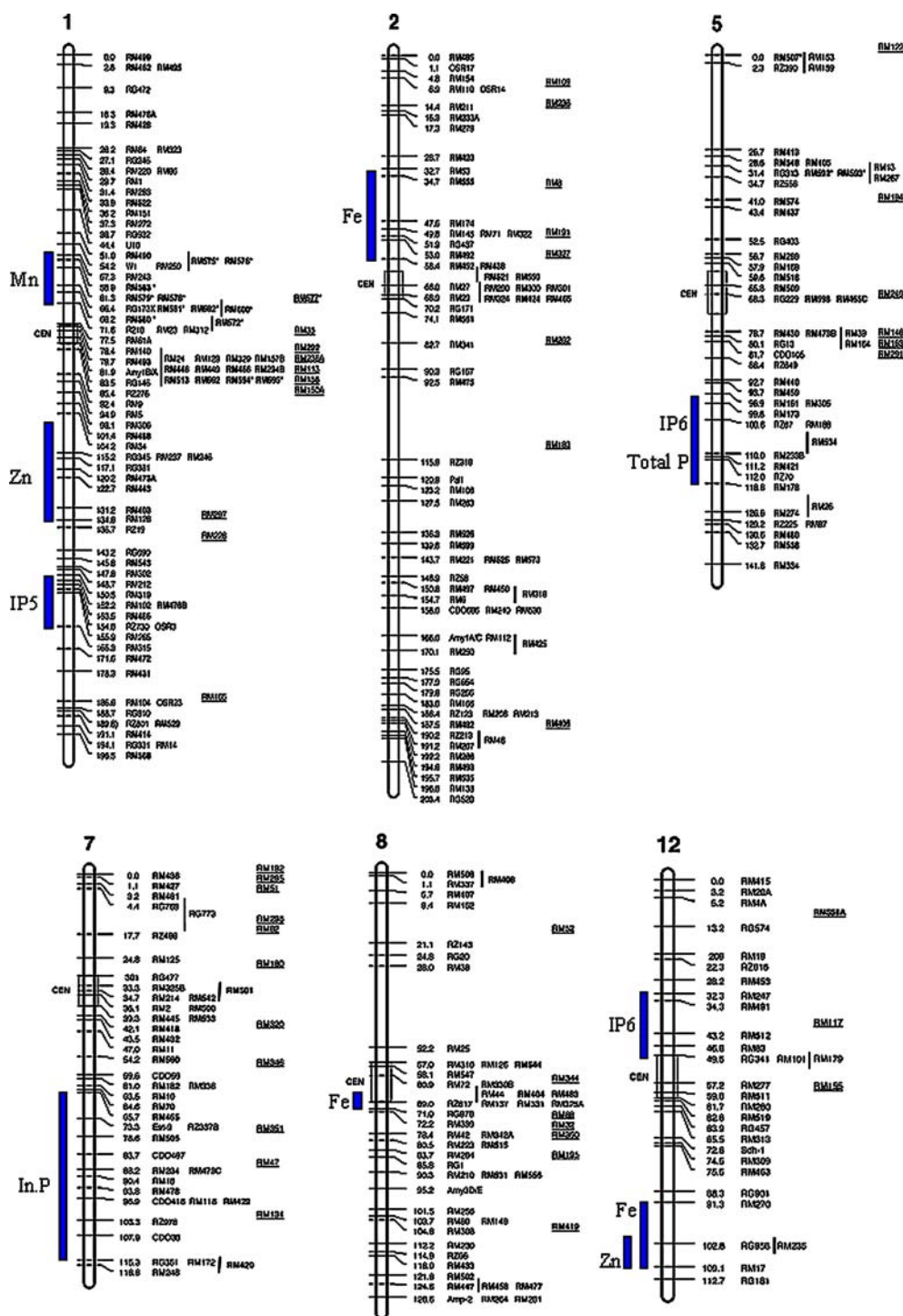


Fig. 2 Chromosome locations of regions associated with grain concentrations of inositol-6-phosphate (IP6), inositol-5-phosphate (IP5), inorganic phosphate, total P, iron, zinc and manganese expressed in double-haploid lines from the

cross IR64 × Azucena based on composite interval mapping. The linkage maps were obtained via the Internet (Temnykh et al. 2001)

Table 2 Quantitative trait loci (QTL) for grain phytate and other nutrients detected by composite interval mapping

Trait	Interval	Chromosome	Flanking markers	LOD ^a	Var. ^b	Add. ^c
Phytate (IP6) (g/kg)	1	5	RM305–RM178	5.6	24.3%	0.514
	2	12	RM247–RM179	3.5	15.4%	0.409
IP5 (g/kg)	1	1	RM102–RM315	4.8	19.8%	–0.041
Inorganic P (g/kg)	1	7	RM70–RM172	5.3	26.5%	0.047
Total P (%)	1	5	RM305–RM178	5.6	24.3%	0.016
Iron (mg/kg)	1	2	RM53–RM300	4.3	16.5%	1.644
	2	8	RM137–RM325A	3.6	18.3%	2.542
	3	12	RM270–RM17	3.8	13.8%	1.456
Zinc (mg/kg)	1	1	RM34–RM237	3.4	15.0%	5.901
	2	12	RM235–RM17	3.1	12.8%	5.407
Manganese (mg/kg)	1	1	RM243–RM312	3.3	16.8%	1.591

^a The LOD score of the peak

^b The proportion of the variance explained by the interval conditioned on the background markers

^c The positive effect indicates that the allele from Azucena has a positive effect on the trait, and the negative effect indicates that the allele from IR64 has a positive effect on the trait

phenotypic variation, respectively. The QTL on chromosome eight showed the largest additive effect (2.5 mg/kg) and was located within a region where a high Fe QTL was previously reported (Gregorio et al. 2000). Zinc concentration also had two QTLs on chromosomes one and 12, explaining 15% and 13% of the total phenotypic variation, respectively. The Zn QTL on chromosome 12 was co-located with the Fe QTL. Manganese concentration also had one QTL on chromosome one, explaining around 17% of the phenotypic variation. Most of the QTLs identified came from the male parent, Azucena, except for the IP5 QTL coming from the female parent, IR64.

The presence of multiple QTLs for phytate, Fe and Zn indicates that these traits are under multi-genic control. Although there were significant phenotypic correlations between phytate and micronutrients, the QTLs of phytate were not located on the same chromosomal regions as those found for Fe, Zn and Mn, suggesting that they were genetically different and thus using molecular markers in breeding and selection would modify the level of phytate without affecting grain micronutrient density. Further evaluation of segregating populations in different environments is needed to validate this result.

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